Purification and Characterization of a Lectin from the Seeds of *Psophocarpus palustris*

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Abstract: A hemagglutinating protein from the phosphate buffered saline (PBS) extract of the seeds of *Psophocarpus palustris* was purified by gel filtration on Sephadex G-150 column. The purified lectin agglutinated specifically red blood cells of human type A and neither type B nor type O. This hemagglutinating activity was inhibited by D-mannose and D-galactose. The purified protein showed one band in non-denaturing polyacrylamide gel electrophoresis (PAGE) as well as in SDS-PAGE. This revealed that the lectin is a homogeneous preparation and a dimeric protein with a molecular weight estimated from gel filtration of 45,000 daltons and subunit molecular weight of 22,700 daltons. Treatment of the lectin with 50 mM EDTA or EGTA had no influence on the hemagglutinating activity but Mg"", Mn"" and Ba"" were effective activators of the purified lectin. The optimal pH for hemagglutination was 7. The purified lectin was stable in pH 7-9 but labile at temperatures over 50°C.

Key words: *Psophocarpus palustris*, lectin, seeds, hemagglutination

INTRODUCTION

Lectins are proteins or glycoproteins usually of plant origin, of non-immunoglobulin in nature, capable of specific recognition of and reversible binding to, carbohydrate moieties of complex glycoconjugates without altering the covalent structure of any of the recognized glycosyl ligands[4,5]. These lectins bind to sugar moieties in cell wall or membranes and thereby change the physiology of the membrane to cause agglutination, mitosis or other biochemical changes in the cell[6]. Lectins are often classified based on saccharide-specificity although a number of them which never showed high affinity to simple saccharides have been found[7].

Lectins have been found to occur in all the classes of organisms from microorganisms to plants and animals[7]. The most abundant source is plants, the best source being the mature seeds of legumes in which they may constitute up to 10% of total proteins[8]. In these leguminous plants and grains, lectins are usually localized in the cotyledon and seed embryos, respectively[9]. They are also found in plant tissues such as leaves, bark and root although in very small amounts where they play important roles in their growth processes[10]. Most plant lectins are storage proteins, which acquire a potential role in defense when the plant or the seed is confronted by insects and fungi[11,12]. Some legume lectins mediate the symbiotic association between leguminous plants and the nitrogen-fixing bacteria[13].

The genus *Psophocarpus* (Leguminous: Papilionaceae) comprises of two species namely *Psophocarpus palustris* and *Psophocarpus monophyllus*.

In this study, we report the isolation and purification of the lectin from mature seeds of *Psophocarpus palustris*, some physicochemical properties of the purified lectin such as blood group and saccharide specificities, molecular size, effect of pH, heat and chelating agents on the hemagglutinating activity.

MATERIALS AND METHODS

Seeds of *Psophocarpus palustris* were obtained from a farm along Ife-Ibadan Road, Ile-Ife, Osun State, Nigeria in November 2003 and were taxonomically identified in the Herbarium of the Department of Botany of the University. Human red blood cells for hemagglutination assays were obtained from healthy individuals with different A, B, O blood group specificities at the Haematology Unit of the University Health Centre and fixed with glutaraldehyde according to the method of Bens et al.[14]. The fixed cells were suspended in Phosphate Buffered Saline (PBS) (0.025 M sodium phosphate, containing 0.15 M NaCl adjusted to pH 7.2 with NaOH solution) containing 0.02% (w/v) sodium azide, to a final concentration of about 4% (v/v) and stored at 4°C.

Preparation of crude extracts: The dried mature seeds were ground into powder and extracted in ten volumes of
50 mM PBS solution, pH 7.2 containing phenyl methyl sulphonyl fluoride (PMSF) (1 mg mL\(^{-1}\)) (to inhibit proteolysis), followed by occasional stirring for more than 12 h according to the method of Adeyemi et al.\(^{18}\) as slightly modified from Animashaun and Hughes\(^{19}\) and Togun et al.\(^{19}\). The solution was then centrifuged at 10,000 rpm for 15 min and the supernatant was brought to 80% saturation with (NH\(_4\))\(_2\)SO\(_4\). The precipitate collected was dissolved in the 50 mM PBS buffer and then dialyzed overnight against the same buffer and stored at -20°C.

**Protein concentration:** Estimation of protein concentration was carried out by the Biuret method of Cornall et al.\(^{19}\) using Bovine Serum Albumin (BSA) as standard.

**Haemagglutination assays:** The haemagglutinating activity of the extract was determined by serial two-fold dilution as described essentially by Kuku and Eretan\(^{19}\), Adeyemi et al.\(^{19}\) and Togun et al.\(^{19}\) using glutaraldehyde-fixed human erythrocytes (types O, A, B) in microtitre plates. Each experiment consisted of 0.1 mL of serially diluted lectin in U-shaped microtitre wells. To each well was added 50 μL of 4% erythrocyte suspension of the different human blood types. The activity was expressed as haemagglutinating titre (U) and is the reciprocal of the last dilution showing visible agglutination of human red blood cells after 1 h of exposure. Specific activity is the activity per mg protein. Values given in this report are the means from three separate measurements.

**Blood group specificity:** Blood group specificity of the extract was established using glutaraldehyde-fixed human erythrocytes from different blood groups of the ABO system as described above.

**Sugar inhibition tests:** To investigate the inhibition of lectin-induced haemagglutination by various sugars, the tests were performed in a manner similar to the haemagglutination test\(^{15-19}\). Serial two-fold dilutions of the extract in PBS were prepared. Each experiment consisted of 0.1 mL of serially diluted lectin in U-shaped microtitre wells. To each well was added 50 μL of 0.2 M sugar solution in PBS using a sugar solution for each row except for the first row which serves as control and 5 μL of 4% suspension of type A red blood cells. The mixture was allowed to incubate for 30 min at room temperature, 50 μL of 2% erythrocyte suspension was added and the mixture left for another 30 min. The haemagglutination titres obtained were compared with a non-sugar containing blank. The test was repeated using the diluted lectin in the presence of various concentrations of a test sugar. The minimal concentration of sugar that fully inhibited hemagglutination was recorded\(^{19}\).

The sugars tested were D-glucose, D-galactose, D-mannose, D-fructose, D-maltose, D-lactose, D-xylene and D-glucosaminehydrochloride.

**Lectin purification:** Three millilitre (28.5 mg mL\(^{-1}\) of protein) of the crude extract was applied to a Sephadex G-150 column (2.5x30 cm) equilibrated with PBS (pH 7.2). 5 mL fractions were collected each at the rate of 20 mL h\(^{-1}\) and the protein concentration and haemagglutinating activity were determined as described earlier. Fractions having haemagglutinating activity were pooled and dialyzed exhaustively against PBS, pH 7.2 at 4°C.

This was purified further by concentrating the pooled fractions and rechromatographing on the Sephadex G-150 column with PBS, pH 7.2

**Determination of molecular weight:** SDS-polyacrylamide gel electrophoresis was carried out as described by Weber and Osborn\(^{13}\) using 7.5% gel concentration. Samples were heated in the sample buffer for 10 min at 100°C. Gels were stained with Coomassie Brilliant blue. The M, standards (Pharmacia) used were bovine serum albumin (67,000), ovalbumin (43,000), thermolysin (37,500), trypsin inhibitor (20,100) and α-lactalbumin (14,400).

Gel filtration for measuring the native molecular weight of lectin was performed on a Sephadex G-150 according to Andrews\(^{20}\). The protein markers (same as above: 3 mg mL\(^{-1}\) of each standard was used) were eluted with PBS, pH 7.2.

**Effect of EDTA and divalent cations:** The effect of divalent cations and chelating agents on haemagglutinating activity was carried out as described by Kuku and Eretan\(^{19}\). The activity of the purified lectin was determined in the presence of various concentrations of MgSO\(_4\), MnCl\(_2\), BaCl\(_2\), and 50 mM EDTA.

**Effect of temperature on haemagglutinating activity:** The effect of temperature on haemagglutinating activity was monitored as described by Sampaio et al.\(^{22}\). Aliquots of the purified lectin were heated for 20 min at 30-80°C. The heated solution was rapidly cooled in ice and assayed for agglutinating activity in comparison with the control which was kept at -20°C. Results were expressed as percentage of the control without heating.

**Effect of pH on haemagglutinating activity:** The effect of pH on haemagglutinating activity was determined by adjusting an aliquot (5 μL) of the purified lectin to different pH values by adding 40 μL of buffer at different
pH (0.2 M citrate-phosphate buffer, pH 3-7; 0.2 M Borate buffer, pH 7.5-9.5; 0.2 M glycine-NaOH buffer, pH 10-12) and 5 μL of erythrocytes following the method of Kuku and Ereman[19]. The final pH of the mixture was estimated with pH paper and hemagglutination titre was recorded. The results were expressed as percentage of the control which was the agglutinating activity of lectin in PBS buffer, pH 7.2.

RESULTS AND DISCUSSION

Potent haemagglutinating activity was detected in the phosphate buffered saline extract of the seeds of *Psophocarpus palustris*. Figure 1 shows the elution profile of *Psophocarpus palustris* seed lectin extract on Sephadex G-1500. The crude extract was resolved into three protein peaks and only one of them showed haemagglutinating activity. On rechromatography on the same resin as shown in Fig. 2, the active peak was further resolved into two broad peaks with only one of them showing activity. The latter peak after gel filtration haemagglutinating protein (*Psophocarpus palustris* Lectin (PPL)) was adjudged pure when only one protein band was observed after electrophoresis on non-SDS-PAGE. The purification procedure yielded approximately 3.0 mg protein with haemagglutinating activity representing a yield of 9.5% and a purification fold of 21 over that of the crude.

PPL agglutinates human erythrocytes of A group only (Table 1). Therefore it is a specific lectin for blood group A. The haemagglutinating activity of the seed extract was inhibited by fructose, maltose, mannose and galactose while xylose, lactose and glucose enhanced the activity (Table 2). Remarkably, it showed no inhibition by glucose, but was inhibited by maltose, a disaccharide composed of two glucose units. It may be that the lectin reacts with a more extended structure than the monosaccharide unit. It has been reported that larger and more complex polysaccharides interact with secondary sites on the lectin surfaces as well as with the primary binding sites[22].

The Mₐ of purified *Psophocarpus palustris* lectin determined by gel filtration was 45,000 daltons. SDS-PAGE showed a protein band with Mₐ 22,700 daltons. It would appear therefore that the native protein is homomeric dimer as in *Dioctea reflexa* agglutinin-II (DRA-II)[23]. The molecular weight of the native lectin is similar to those of *Trichosanthes anguina* seeds, 45,000 daltons[20], *Artocarpus integrifolia*, 46,000 daltons and *Maclea pomifera*, 44,000 daltons[25].

![Fig. 1: Elution profile of the purification of *Psophocarpus palustris* on Sephadex G-150 column (2.5x30 cm). The column was eluted with phosphate buffer saline, pH 7.2 at a flow rate of 10 mL h⁻¹. Five milliliter fractions were collected and monitored for protein and agglutination. x-x (agglutination titre) and o-o (OD₅₄₀).](image1)

![Fig. 2: Re-chromatography of post gel filtration fraction on Sephadex G-150 column (2.5x30 cm). Eluant was PBS, pH 7.2. The flow rate was 20 mL h⁻¹ and the fraction size was 5 mL. x-x (agglutination titre) and o-o (OD₅₄₀).](image2)
Fig. 3: pH dependence of heamagglutinating activity. The pH was ranged between 3 and 12 using three different buffers as described in the text.

Chelating agents such as EDTA and EGTA at 50 mM had no effect on the agglutinating activity. However, the activity was enhanced by divalent cations although no direct determination of the presence, identity and amount of divalent metal ions in the native protein was carried out. Also, the heamagglutinating activity of *Psophocarpus palustris lectin* rapidly declined when the lectin was heated above 50°C, activity was reduced to half at 60°C and was completely lost at 70°C, an indication that its activity depends on the native conformation of the protein. This finding suggests that the activity of the lectin is related to cations just like the metal ions in Concanavalin A which protect it from proteolytic and temperature degradation[^1][^2]. Because metal ions bind strongly to lectins, treatment with EDTA fails to remove cations[^2].

The hemagglutinating activity of the lectin purified from *Psophocarpus palustris* seeds is pH dependent. The lectin was stable in the pH range from 7 to 9 (Fig. 3), more acidic or basic pHs decreased both stability and activity. This result is very similar to the behaviour of other lectins reported in the literature[^1][^2][^3]. For example, the lectin from *Parkia javanica* beans also depend on pH, the optimal pH value was 7, it was stable in the pH range 7-10 and more acidic or basic pHs decreased both stability and activity[^3]. The lectin of *P. filicina* was also stable in the pH range 4-9, retaining 50% of its activity at pH 3 and 10 and 25% of activity at pH 11-12[^3]. It is possible that changes in the ionization state with an increase in pH may lead to a weaker binding of the metal ions, which are apparently required for the maintenance of structure required for maximal activity (*Vide supra*). pH dependence, which is observed in virtually all enzyme reactions, is a consequence of the protein composition. Numerous ionizable groups at the surface of the protein molecule and the active center are capable of reacting with H⁺ or OH⁻. Any change of pH is therefore associated with a change in the ionization state of the molecule, which in turn, determines the binding forces between enzyme and substrate. It is also possible that increase in OH⁻ ions caused a change in the ionization state of the lectin thereby affecting the binding forces between the lectin and the erythrocyte membrane that eventually led to loss of activity.

**REFERENCES**


